



sequence listing.ST25.txt
SEQUENCE LISTING

<110> Cano, Carlos Antonio Durante
Nieto, Enrique Gerardo Guillen
Acosta, Anabel Alvarez
Munoz, Luis Emilio Carpio
Vazquez, Diogenes Quintana
Rodriguez, Carmen Elena Gomez
Rodriguez, Recardo de la Caridad Siva
Galvez, Consuelo Nazabal
Angulo, Maria de Jesus Leal
Dunn, Alejandro Miguel Martin

<120> Expression System of Heterologous Antigens as Fusion Proteins

<130> LEXSA P-13DIV2

<140> 09/612,925

<141> 2000-07-10

<150> US 08/930,917

<151> 1997-09-16

<150> PCT/CU97/00001

<151> 1997-01-17

<160> 31

<170> PatentIn version 3.2

<210> 1

<211> 1797

<212> DNA

<213> *Neisseria meningitidis* (group B)

<400> 1
atgctagata aaagaatggc tttagttgaa ttgaaagtgc ccgacattgg cggacacgaa 60
aatgttagata ttatcgccgt tgaagtaaac gtgggcgaca ctattgctgt ggacgatacc 120
ctgattactt tggaaaccga taaagcgact atggacgtac ctgctgaagt tgcaggccta 180
gtcaaagaag ttaaagttaa agtcggcgac aaaatctctg aagggtggttt gattgtcgac 240
gttgaagctg aaggcacggc agccgctcct aaagccgaag cggctgccgc cccggcgcaa 300
gaagccccta aagctgccgc tcctgctccg caagccgcgc aattcggcgg ttctgcccgt 360
gccgagtacg acgtggtcgt attgggtggc ggtcccgccg gttactccgc tgcatttgcc 420
cctgcccgtg aaggcttgaa agtcgcccattc gtcgaacgtt acaaaacttt gggcggcggtt 480
tgcctgaacg tcggctgtat cccttccaaa gccttgttc acaatgccgc cgttatcgac 540
gaagtgcgcc acttggctgc caacggtatac aaatacccg agccggaact cgacatcgat 600
atgcttcgcg cctacaaaga cggcgtagtt tcccgccctca cggccggttt ggcaggtatg 660
gcgaaaagcc gtaaagtggc cgttatccaa ggcgacgggc aattcttaga tccgcaccac 720
ttggaagtgt cgctgactgc cggcgacgcg tacgaacagg cagcccctac cggcgagaaa 780
aaaatcggtt ccttcaaaaa ctgtatcatt gcagcaggca gccgcgtaac caaactgcct 840

sequence listing.ST25.txt

ttcattcctg aagatccgca catcatcgat tccagcggcg cattggctct gaaagaagta	900
ccgggcaaac tgctgattat cggcggcgcc attatcagcc tcgagatggg tacggttac	960
agcacgctgg gttcgcgttt ggatgtggtt gaaatgatgg acggcctgat gcaaggcgca	1020
gaccgcgatt tggtaaaagt atggcaaaaa caaaacgaat accgtttga caacattatg	1080
gtcaacacca aaaccgttgc agttgagccg aaagaagacg gcgttacgt tacctttgaa	1140
ggcgcgaacg cgcctaaaga gccgcaacgc tacgatgccg tattgggtgc cgccggccgc	1200
gcccacg gcaaactcat cagcgcggaa aaagcaggcg ttgccgtaac cgatcgcggc	1260
ttcatcgaag tggacaaaca aatgcgtacc aatgtgccgc acatctacgc catcggcgac	1320
atcgtcggtc agccgatgtt ggcgcacaaa gccgttacg aaggccacgt tgccgccgaa	1380
aactgcgccg gccacaaagc ctacttcgac gcacgcgtga ttccggcgt tgcctacact	1440
tccccgaag tggcgtgggt gggcgaacc gaaactgtccg ccaaagcctc cggccgcaaa	1500
atcaccaaag ccaacttccc gtggcggct tccggccgtg cgattgccaa cgggtgcac	1560
aacggcttta ccaagctgat ttttgcgtt gaaaccggcc gcatcatcg cgccggcatt	1620
gtcggtccga acggtggcga tatgatccgc gaagtctgcc ttgccatcga aatgggctgc	1680
gacgcggcag acatcggcaa aaccatccac ccgcacccga ccttgggcga atccatcggt	1740
atggcggcgg aagtggcatt gggtacttgt accgacctgc ctccgcaaaa gaaaaaa	1797

<210> 2

<211> 47

<212> PRT

<213> *Neisseria meningitidis* (group B)

<400> 2

Met Val Asp Lys Arg Met Ala Leu Val Glu Leu Lys Val Pro Asp Ile	
1 5 10 15	

Gly Gly His Glu Asn Val Asp Ile Ile Ala Val Glu Val Asn Val Gly	
20 25 30	

Asp Thr Ile Ala Val Asp Asp Thr Leu Ile Thr Leu Asp Leu Glu	
35 40 45	

<210> 3

<211> 146

<212> DNA

<213> *Neisseria meningitidis* (group B)

<400> 3

ttccatggta gataaaagaa tggcttagt tgaattgaaa gtgcccgaca ttggcggaca	60
cgaaaatgta gatattatcg cggttgaagt aaacgtggc gacactattg ctgtggacga	120

<210> 4
 <211> 18
 <212> PRT
 <213> *Neisseria meningitidis* (group B)

<400> 4

Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile Thr Leu
 1 5 10 15

Asp Leu

<210> 5
 <211> 18
 <212> PRT
 <213> *Neisseria meningitidis* (group B)

<400> 5

Val Glu Val Gly Ser Lys Ile Tyr Val Asp Asp Gly Leu Ile Ser Leu
 1 5 10 15

Gln Val

<210> 6
 <211> 32
 <212> PRT
 <213> *Neisseria meningitidis* (group B)

<400> 6

Leu Val Glu Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp
 1 5 10 15

Ile Ile Ala Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp
 20 25 30

<210> 7
 <211> 32
 <212> PRT
 <213> *Neisseria meningitidis* (group B)

<400> 7

Leu Arg Glu Val Gln Val Pro Asp Arg Lys Leu His Lys Gly Val Gln
 1 5 10 15

Leu Leu Ala Gly Glu Leu Gly Ile Gly Glu Ala Leu Gln Val Asp Asp
 20 25 30

sequence listing.ST25.txt

<210> 8

<211> 162

<212> PRT

<213> Neisseria meningitidis (group B)

<400> 8

Met Val Asp Lys Arg Met Ala Leu Val Glu Leu Lys Val Pro Asp Ile
1 5 10 15

Gly Gly His Glu Asn Val Asp Ile Ile Ala Val Glu Val Asn Val Gly
20 25 30

Asp Thr Ile Ala Val Asp Asp Thr Leu Ile Thr Leu Asp Leu Asp Ser
35 40 45

Arg Gly Ile Arg Ile Gly Pro Gly Arg Ala Ile Leu Ala Thr Ala Gly
50 55 60

Gly Gly Ala Arg Gln Ser Thr Pro Ile Gly Leu Gly Gly Ala Leu Tyr
65 70 75 80

Thr Thr Ala Gly Gly Gly Ala Arg Lys Ser Ile Thr Lys Gly Pro Gly
85 90 95

Arg Val Ile Tyr Ala Thr Ala Gly Gly Gly Ala Arg Lys Arg Ile His
100 105 110

Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Ala Gly Gly Gly Ala Arg
115 120 125

Lys Arg Ile Thr Met Gly Pro Gly Arg Val Tyr Tyr Thr Thr Ala Gly
130 135 140

Gly Gly Ala Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val
145 150 155 160

Thr Ile

<210> 9

<211> 489

<212> DNA

<213> Neisseria meningitidis (group B)

<400> 9

atggtagata aaagaatggc ttttagttgaa ttgaaagtgc ccgacattgg cggacacgaa 60

aatgttagata ttatcgccgt tgaagtaaac gtgggcgaca ctattgctgt ggacgataacc 120

ctgattactt tggatctaga ctcgagaggc attcgtatcg gcccaggctcg cgcaattta 180

sequence listing.ST25.txt
gcaacagctg gcgggtggcgc acgtcaatct acccctattg gtttaggtca ggctctgtat 240
acgactgccc gcgggtggtgc gcgcaaaagt atcaccaagg gtccaggccg cgtcatttac 300
gccaccgcgg gcggcgggtgc ccgtaagcgt atccacattg gcccaggccg tgcattctat 360
actacagcag gtgggtggcgc acgtaaacgc atcactatgg gtcctggtcg cgtctattac 420
acgaccgcgt gcggcgggtgc tagcattcgc atccaacgcg gccctggtcg tgcatttgt 480
accatatga 489

<210> 10
<211> 47
<212> PRT

<213> *Neisseria meningitidis* (group B)

<400> 10

Met Leu Asp Lys Arg Met Ala Leu Val Glu Leu Lys Val Pro Asp Ile
1 5 10 15

Gly Gly His Glu Asn Val Asp Ile Ile Ala Val Glu Val Asn Val Gly
20 25 30

Asp Thr Ile Ala Val Asp Asp Thr Leu Ile Thr Leu Glu Thr Asp
35 40 45

<210> 11
<211> 27
<212> DNA
<213> Artificial

<220>
<223> Primer 5' No. 1573

<400> 11
ttccatggta gatamagmtg gctttag 27

<210> 12
<211> 29
<212> DNA
<213> Artificial

<220>
<223> Primer 3' No. 1575

<400> 12
tttctagatc caaagtaatc aggatatcg 29

<210> 13
<211> 26
<212> DNA
<213> Artificial

<220>
<223> Primer 3' No. 2192

sequence listing.ST25.txt

<400> 13
ggcggttctg ccgattaagg atccga

26

<210> 14
<211> 146
<212> DNA
<213> Artificial

<220>
<223> Derived fragment from the first 47 amino acids of the P64k antigen of *N. meningitidis*. The restriction sites Ncol (positions 3 to 8) and XbaI (positions 139 to 144) are introduced by PCR, which provokes changes in the nucleotide sequence of this

<400> 14
ttccatggta gataaaagaa tggcttagt tgaattgaaa gtgcccaca ttggcggaca 60
cgaaaatgta gatattatcg cggttgaagt aaacgtgggc gacactattg ctgtggacga 120
taccctgatt actttggatc tagaaa 146

<210> 15
<211> 47
<212> PRT
<213> Artificial

<220>
<223> Stabilizer fragment derived from the first 47 amino acids of the P64k antigen of *N. meningitidis*

<400> 15

Met Val Asp Lys Arg Met Ala Leu Val Glu Leu Lys Val Pro Asp Ile
1 5 10 15

Gly Gly His Glu Asn Val Asp Ile Ile Ala Val Glu Val Asn Val Gly
20 25 30

Asp Thr Ile Ala Val Asp Asp Thr Leu Ile Thr Leu Asp Leu Glu
35 40 45

<210> 16
<211> 16
<212> DNA
<213> Artificial

<220>
<223> Oligonucleotide used to introduce restriction sites XbaI, EcoV, and BamHI in the 3' end of the stabilizer fragment of SEQ. ID. NO. 13

<400> 16
ctagatttga tatcag 16

<210> 17

sequence listing.ST25.txt

<211> 16
<212> DNA
<213> Artificial

<220>
<223> Oligonucleotide used to introduce restriction sites XbaI, EcoV, and BamHI in the 3' end of the stabilizer fragment of SEQ. ID. NO. 13

<400> 17
gatcctgata tcaaatt

16

<210> 18
<211> 15
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 18

Ser Arg Gly Ile Arg Ile Gly Pro Gly Arg Ala Ile Leu Ala Thr
1 5 10 15

<210> 19
<211> 15
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 19

Arg Gln Ser Thr Pro Ile Gly Leu Gly Gln Ala Leu Tyr Thr Thr
1 5 10 15

<210> 20
<211> 15
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 20

Arg Lys Ser Ile Thr Lys Gly Pro Gly Arg Val Ile Tyr Ala Thr
1 5 10 15

<210> 21
<211> 15
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 21

Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
1 5 10 15

<210> 22
<211> 15
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 22

sequence listing.ST25.txt

Arg Lys Arg Ile Thr Met Gly Pro Gly Arg Val Tyr Tyr Thr Thr
1 5 10 15

<210> 23
<211> 15
<212> PRT
<213> *Neisseria meningitidis* (group B)
<400> 23

Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile
1 5 10 15

<210> 24
<211> 15
<212> PRT
<213> Human immunodeficiency virus type 1
<400> 24

Thr Ser Ile Thr Ile Gly Pro Gly Gln Val Phe Tyr Arg Thr Gly
1 5 10 15

<210> 25
<211> 15
<212> PRT
<213> Human immunodeficiency virus type 1
<400> 25

Arg Gln Arg Thr Ser Ile Gly Gln Gly Gln Ala Leu Tyr Thr Thr
1 5 10 15

<210> 26
<211> 5
<212> PRT
<213> Artificial
<220>
<223> Spacer peptide that divides the various V3 epitopes in the MEPS
TAB3, TAB4, TAB9, and TAB13
<400> 26

Ala Gly Gly Gly Ala
1 5

<210> 27
<211> 141
<212> PRT
<213> Artificial
<220>
<223> Multiepitopic polypeptides that includes several copies of the
central part of the variable region 3 of the gp120 protein of the
HIV-1

sequence listing.ST25.txt

<400> 27

Met Ala Pro Thr Ser Ser Ser Thr Ala Gln Thr Gln Leu Gln Leu Glu
1 5 10 15

His Leu Leu Leu Asp Leu Gln Ile Phe Leu Ser Arg Gly Ile Arg Ile
20 25 30

Gly Pro Gly Arg Ala Ile Leu Ala Thr Ala Gly Gly Gly Ala Arg Gln
35 40 45

Ser Thr Pro Ile Gly Leu Gly Gly Ala Leu Tyr Thr Thr Ala Gly Gly
50 55 60

Gly Ala Arg Lys Ser Ile Thr Lys Gly Pro Gly Arg Val Ile Tyr Ala
65 70 75 80

Thr Ala Gly Gly Ala Arg Lys Arg Ile His Ile Gly Pro Gly Arg
85 90 95

Ala Phe Tyr Thr Thr Ala Gly Gly Gly Ala Arg Lys Arg Ile Thr Met
100 105 110

Gly Pro Gly Arg Val Tyr Tyr Thr Thr Ala Gly Gly Gly Ala Ser Ile
115 120 125

Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile
130 135 140

<210> 28

<211> 162

<212> PRT

<213> Artificial

<220>

<223> Multiepitopic polypeptides that includes several copies of the central part of the variable region 3 of the gp120 protein of the HIV-1

<400> 28

Met Val Asp Lys Arg Met Ala Leu Val Glu Leu Lys Val Pro Asp Ile
1 5 10 15

Gly Gly His Glu Asn Val Asp Ile Ile Ala Val Glu Val Asn Val Gly
20 25 30

Asp Thr Ile Ala Val Asp Asp Thr Leu Ile Thr Leu Asp Leu Asp Ser
35 40 45

Arg Gly Ile Arg Ile Gly Pro Gly Arg Ala Ile Leu Ala Thr Ala Gly
Page 9

50

55

sequence listing.ST25.txt

60

Gly Gly Ala Arg Gln Ser Thr Pro Ile Gly Leu Gly Gly Ala Leu Tyr
65 70 75 80

Thr Thr Ala Gly Gly Gly Ala Arg Lys Ser Ile Thr Lys Gly Pro Gly
85 90 95

Arg Val Ile Tyr Ala Thr Ala Gly Gly Gly Ala Arg Lys Arg Ile His
100 105 110

Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Ala Gly Gly Gly Ala Arg
115 120 125

Lys Arg Ile Thr Met Gly Pro Gly Arg Val Tyr Tyr Thr Thr Ala Gly
130 135 140

Gly Gly Ala Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val
145 150 155 160

Thr Ile

<210> 29

<211> 202

<212> PRT

<213> Artificial

<220>

<223> Multiepitopic polypeptides that include several copies of the central part of the variable region 3 of the gp120 protein of the HIV-1

<400> 29

Met Val Asp Lys Arg Met Ala Leu Val Glu Leu Lys Val Pro Asp Ile
1 5 10 15

Gly Gly His Glu Asn Val Asp Ile Ile Ala Val Glu Val Asn Val Gly
20 25 30

Asp Thr Ile Ala Val Asp Asp Thr Leu Ile Thr Leu Asp Leu Asp Ser
35 40 45

Arg Gly Ile Arg Ile Gly Pro Gly Arg Ala Ile Leu Ala Thr Ala Gly
50 55 60

Gly Gly Ala Arg Gln Ser Thr Pro Ile Gly Leu Gly Gln Ala Leu Tyr
65 70 75 80

sequence listing.ST25.txt

Thr Thr Ala Gly Gly Ala Arg Lys Ser Ile Thr Lys Gly Pro Gly
85 90 95

Arg Val Ile Tyr Ala Thr Ala Gly Gly Gly Ala Arg Lys Arg Ile His
100 105 110

Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Ala Gly Gly Gly Ala Arg
115 120 125

Lys Arg Ile Thr Met Gly Pro Gly Arg Val Tyr Tyr Thr Thr Ala Gly
130 135 140

Gly Gly Ala Arg Gln Arg Thr Ser Ile Gly Gln Gly Gln Ala Leu Tyr
145 150 155 160

Thr Thr Ala Gly Gly Ala Thr Ser Ile Thr Ile Gly Pro Gly Gln
165 170 175

Val Phe Tyr Arg Thr Gly Ala Gly Gly Gly Ala Ser Ile Arg Ile Gln
180 185 190

Arg Gly Pro Gly Arg Ala Phe Val Thr Ile
195 200

<210> 30
<211> 368
<212> DNA
<213> Artificial

<220>
<223> Synthetic fragment that codifies for MEP TAB9. Restriction sites XbaI and BamHI are introduced.

<400> 30
tctagactcg agaggcattc gtatcgcccc aggtcgcgca attttagcaa cagctggcgg 60
tggcgcacgt caatctaccc ctattggttt aggtcaggct ctgtatacga ctgccggcgg 120
tggtgccgcgc aaaagtatca ccaagggtcc aggccgcgtc atttacgcca ccgcggggcgg 180
cggtgcccgta aagcgtatcc acattggccc aggccgtgca ttctatacta cagcaggtgg 240
tggcgcacgt aaacgcatac caatgggtcc tggtcgcgtc tattacacga ccgcgtggcgg 300
cggtgctagc attcgcatcc aacgcggccc tggtcgtgca tttgtgacca tatgataacg 360
cgggatcc 368

<210> 31
<211> 5
<212> PRT
<213> Neisseria meningitidis (group B)

<400> 31

sequence listing.ST25.txt

Met Leu Asp Lys Arg
1 5